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DETERMINATION OF MS2 BACTERIOPHAGE STABILITY AT HIGH pH USING THE INTEGRATED VIRUS DETECTION SYSTEM (IVDS)

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14. ABSTRACT
A sample of MS2 stock solution was prepared by filtering with reverse osmosis water in a cross flow ultra filtration system. The cross flow ultra filtration system, which uses a molecular weight cut-off (MWCO) filter of 100K Daltons, circulates the MS2 stock solution with the water. The filter allows any impurities (e.g. growth media, miscellaneous salts, and proteins) to pass through the filter but retain the MS2. The sample of MS2 is concentrated and retained for testing at high pH to determine the survival rate. The treated MS2 samples were analyzed using the Integrated Virus Detection System (IVDS). The detection stage of the IVDS consists of an electrospray unit to inject samples into the detector, a Differential Mobility Analyzer and a Condensate Particle Counter.

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PREFACE

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Acknowledgments

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CONTENTS

FIGURES

TABLE

DETERMINATION OF MS2 BACTERIOPHAGE STABILITY AT HIGH pH USING THE INTEGRATED VIRUS DETECTION SYSTEM (IVDS)

1. INTRODUCTION

The stability of viruses under different environmental conditions has always been a problem for microbiologists. Measuring this stability requires subjecting the virus to a harsh environment and monitoring the decay of the number concentration of virus particles with time. Until recently, measuring this stability had been exceedingly difficult. However, a new device - the Integrated Virus Detection System (IVDS), which can characterize and measure the number concentration of viruses, has been developed at the U.S. Army Edgewood Chemical Biological Center (ECBC). The IVDS relies on measuring physical characteristics (size) of the virus instead of bacteriological means. This allows us to measure the number concentration of virus particles quickly. The IVDS is described in ECBC-TR-018.[1] This report is the first in a series of reports that describes studies on the survivability of MS2 bacteriophage under different environmental conditions.

2. EXPERIMENTAL PROCEDURES

A sample of MS2 stock solution (2 mL) was obtained from Dr. Deborah Kuzmanovic, National Institute of Standards and Technology (NIST), Gaithersburg, MD. The stock solution was diluted with 100 mL of distilled water and filtered by the Ultra Filtration (UF) subsystem of the IVDS using 100K-Da filters (for details on the IVDS and its different subsystems, the reader is referred to ECBC-TR-018 and ECBC-TR-463.[2]

The filtration system removes any material with a molecular weight smaller than the filtration system is set for (in this case, 100K-Da), such as growth media, salt molecules, and proteins from the solution and leaves a concentrated virus solution. The concentrated MS2 solution was added to 23 mL of 20 mM ammonium acetate solution. The ammonium acetate is needed to increase the conductivity of the solution to allow it to be injected into the test module of the IVDS. The MS2 solution was concentrated again by the UF subsystem to a total of 2.5 mL of clean solution. The bacteriophage solution was then subjected to high pH to determine the survival rate.

A high pH solution was prepared by mixing 1:1 solutions of 1 M 2-aminoethanol and 20 mM ammonium acetate. The resultant solution had a pH of 11.1. For testing, 10 μl of the concentrated MS2 was mixed with 10 μl of the high pH solution, and the number concentration of virus particles was measured over time.

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[1]Wick, Charles H.; Anderson, David M.; McCubbin, Patrick E. Characterization of the Integrated Virus Detection System (IVDS) using MS-2 Bacteriophage; ECBC-TR-018; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, 1999; UNCLASSIFIED Report (AD-A364 117).

[2]Wick, Charles H.; McCubbin, Patrick E.; Birenzvige, A. Detection and Identification of Viruses using the Integrated Virus Detection System (IVDS); ECBC-TR-463; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, November 2005.

3. RESULTS AND DISCUSSION

The analysis results of the initial purified and concentrated sample of MS2 are shown in Figure 1. The resultant graph shows a typical MS2 peak at 22.3 nm.[2] The counts were numerically divided by a factor of two to allow graphical comparison with the high pH solutions. Figure 2 provides the analysis results immediately after mixing the MS2 solution with the high pH solution. Subsequent analyses were conducted after 16 hr and after 42 hr. These results are shown in Figures 3 and 4, respectively.

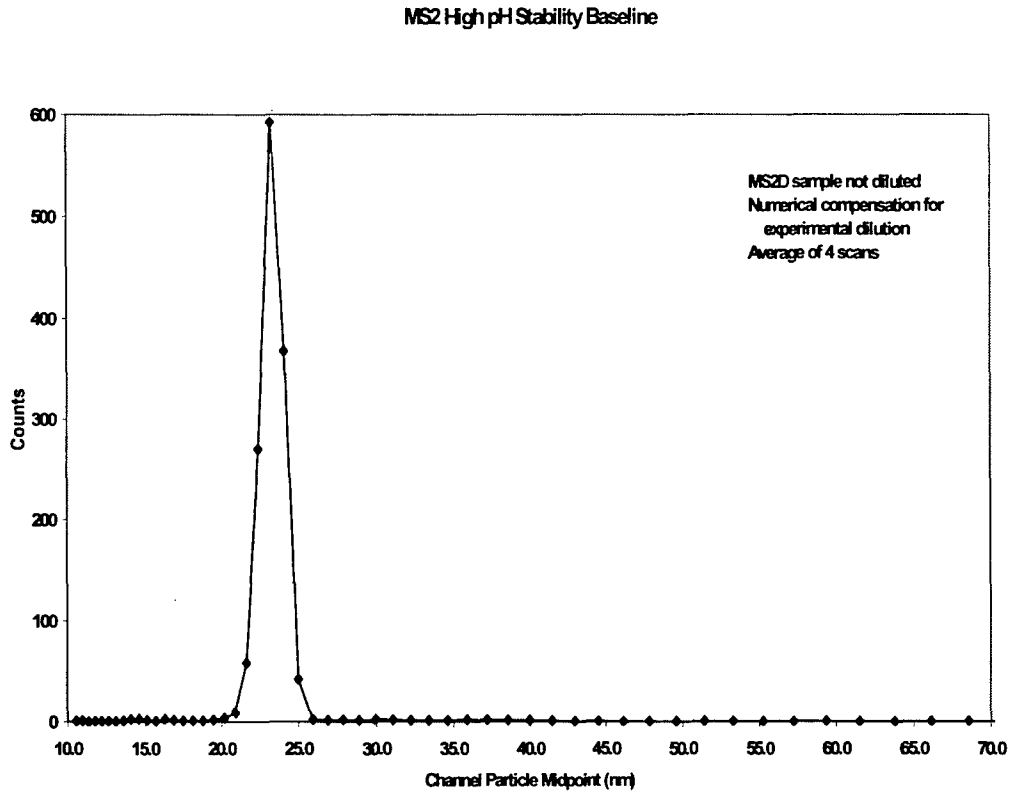


Figure 1: Base Line MS2 Count

[2]Wick, Charles H.; McCubbin, Patrick E.; Birenzvige, A. Detection and Identification of Viruses using the Integrated Virus Detection System (IVDS); ECBC-TR-463; U.S. Army Edgewood Chemical Biological Center: Aberdeen Proving Ground, MD, November 2005.

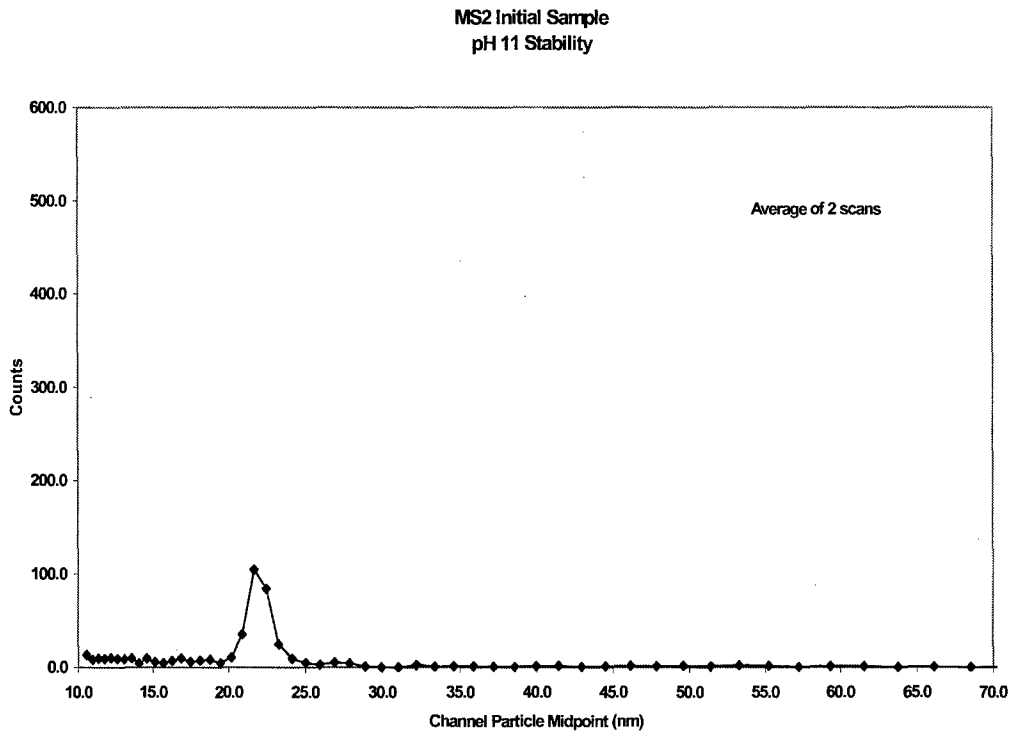


Figure 2: MS-2 Count Immediately Following Addition of the High pH Solution

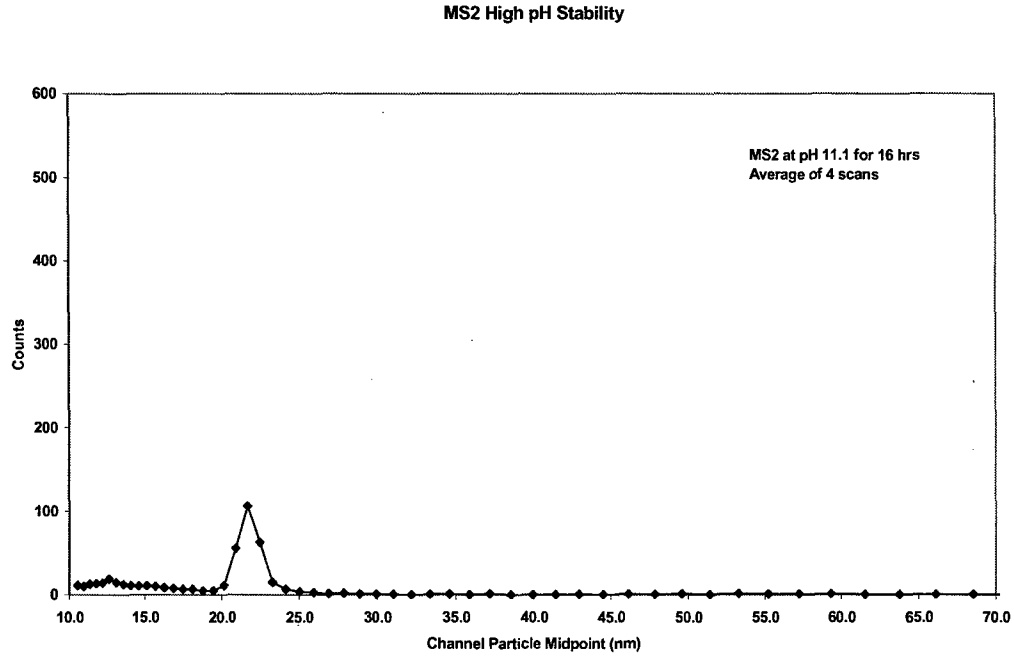


Figure 3: MS2 Count in High pH Solution after 16 hr

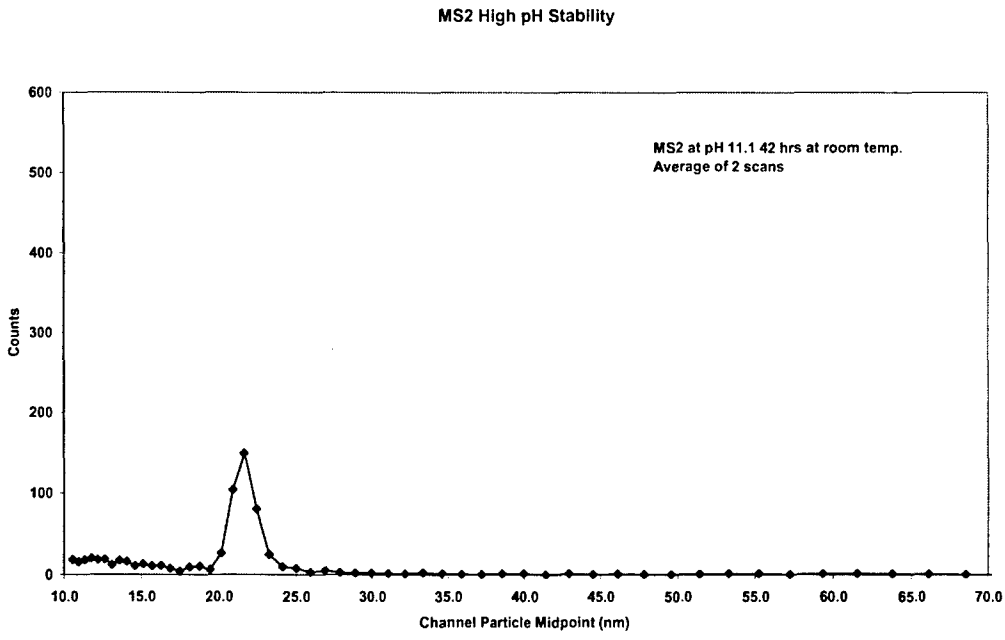


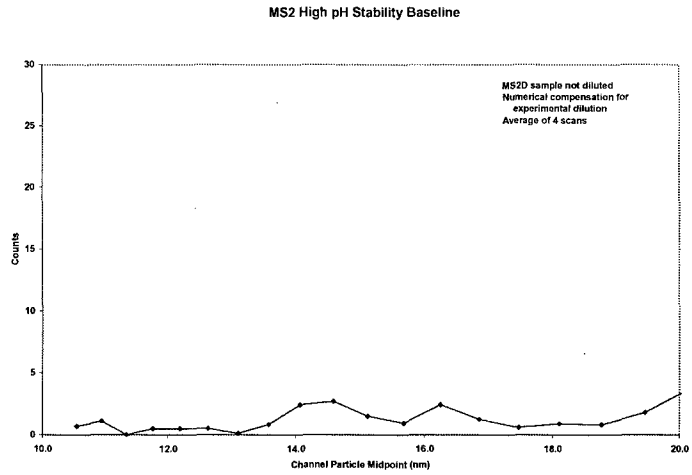
Figure 4: MS2 Count in High pH Solution after 42 hr

Comparing Figures 1 and 2, the number concentration of virus particles drops from almost 800 to about 140 immediately upon increasing the pH. However, after 16 hr at a highly alkaline solution, the concentration remains the same and even shows a sign of increasing after 42 hr. The results are tabulated in the following table.

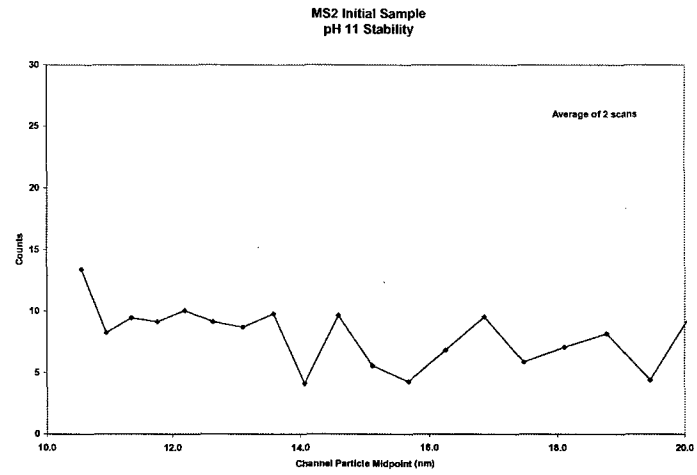
Table. Average Particle Count of MS2 Scans

	10.55 to 19.46 nm	20.17 to 29.96 nm	Comments
Baseline	20 ± 0.8	1355 ± 194	Initial dilution 1:1, numerical compensation to a 1:2 experimental dilution
Initial sample	144 ± 2.4	286 ± 35	Diluted 50:50 from baseline
16 hr at pH 11.1	189 ± 3.7	268 ± 34	Diluted 50:50 from baseline
42 hr at pH 11.1	237 ± 4.7	417 ± 49	Diluted 50:50 from baseline

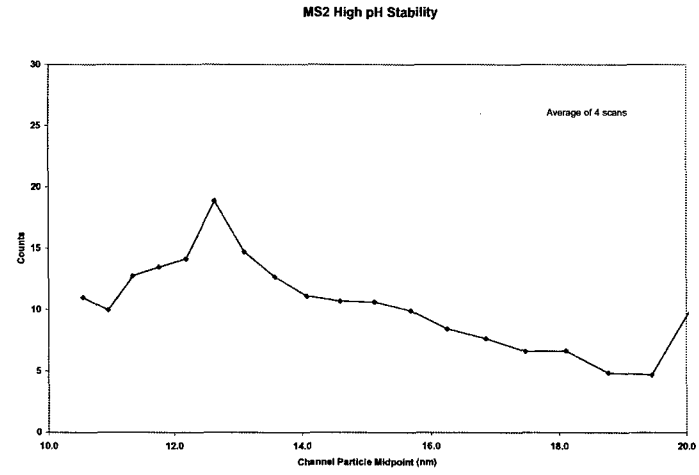
As the concentration of viruses declines, we can expect that the cell material and the core of the virus will disintegrate to lower sized protein particles. This is indeed what happens as can be observed in Figure 5. A description of proteins and constituents of the MS2 bacteriophage is included in the Appendix.



a. MS2 Sample at Neutral pH

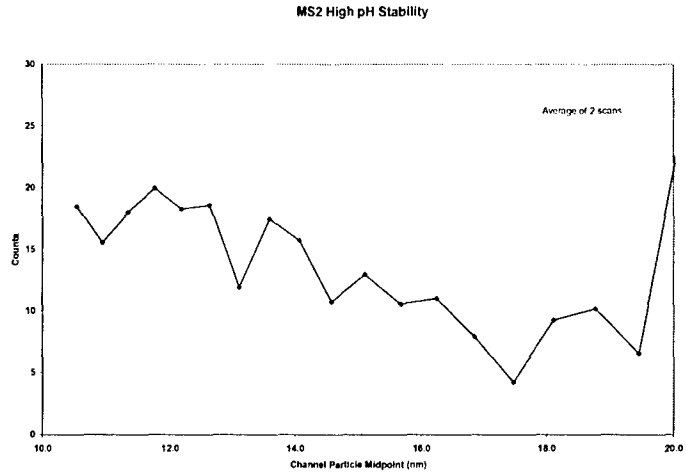


b. MS2 Sample Immediately after Increasing the pH



c. MS2 Sample 16 hr after Increasing the pH

Figure 5. IVDS Scan of Particles Smaller than 20 nm



d. MS2 Sample 42 hr after Increasing the pH

Figure 5: IVDS Scan of Particles Smaller than 20 nm (continued)

4. CONCLUSIONS

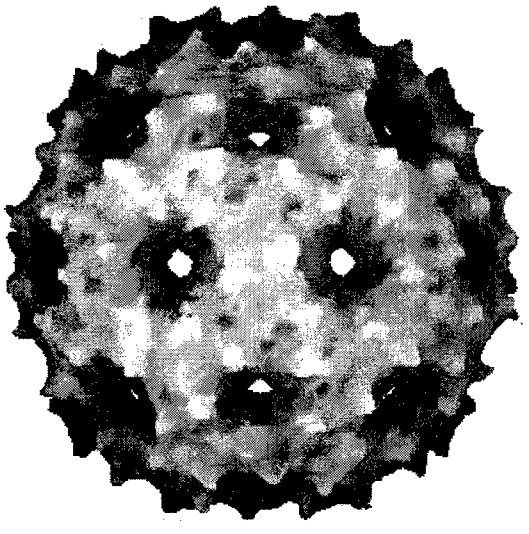
Using the Integrated Virus Detection System, we were able to show that high pH causes a breakdown of viruses. Initially, upon increase of the pH from neutral to highly basic, the number concentration of virus particles diminishes significantly. However, no further degradation with time is observed. The degradation of viruses is accompanied by increased concentration of protein, which are break-down materials of the cell and core of the virus.

APPENDIX

DESCRIPTION OF THE MS2 VIRUS AND ITS COMPONENTS

Virus	Type	Proteins	Size	Comments
Leviviridae	MS2 bacteriophage	Two structural virion proteins found. Protein size 35000-44000 Da. Capsid contains one copy of A protein, which is required for maturation of the virion and for pilus attachment. Protein size of 2nd largest 14000 Da. Coat protein; capsid contains 180 copies of the coat protein arranged in 60 identical triangular units.	Virions not enveloped. Nucleocapsids isometric; 24-26 nm in diameter. Symmetry icosahedral. 32 capsomers per nucleocapsid.	Molecular mass (Mr) of virion 3.6-4.2 x 10^6 (depending on the genus). Buoyant density 1.46 g cm^{-3} in CsCl. Sedimentation coefficient 80-84 S. Virions sensitive to detergents. Virions not sensitive by di-ethyl ether and chloroform. Infectivity reduced after exposure to irradiation.

PDB-ID : 2MS2
Resolution : 2.8 Å
A.A.Seq.Acc.# : P03612
Family :Leviviridae
T Number : 3
# of Subunits : 180
Diameter: Ave:268Å; Max:288Å



Rendered Surface

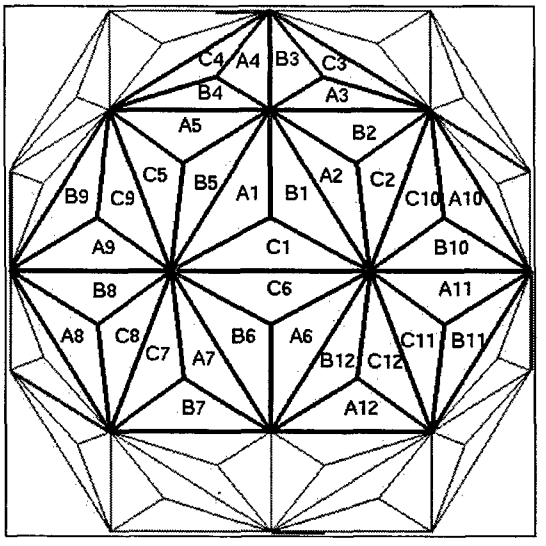
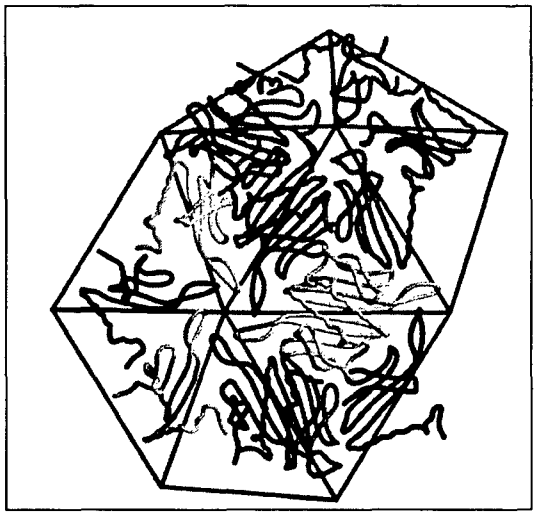
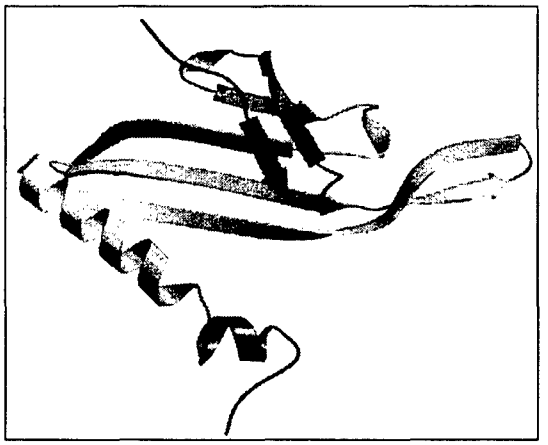


Diagram of the tertiary folding of constituent subunits in MS2. T= 3 Lattice



MS2 Capsomeres



N-terminus (blue) to C-terminus (red).
MS2 protein subunits.

Source: Reddy et al. Virus Particle Explorer (VIPER), a Website for virus capsid structures and their computational analysis. *J. Virol.* **2001** *75*, pp 11943-11947.